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Research Article

COMPARATIVE STUDY OF INHIBITION OF DRUG POTENCIES OF TYROSINE KINASE INHIBITORS: A COMPUTATIONAL AND MOLECULAR DOCKING STUDY

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ABSTRACT

The macromolecular structure reveals the molecular basis for insulin receptor activation via autophosphorylation, and provides insights into tyrosine kinase inhibitor specificity and the mechanism of phosphotransfer. The ligand binding site of intracellular C-terminal region displays the highest level of conservation and comprises catalytic domains responsible for the kinase activity of these receptors, which catalyses receptor autophosphorylation and tyrosine phosphorylation of RTK substrates. This work reports the analysis of series of compounds through virtual screening approach with Insulin receptor tyrosine kinase. In this present study states that, the library of compounds constructed based on heterocyclic ring and performed docking using GLIDE, in an order with inhibition scores and compared with already existing drugs. It leads to the identification of potential inhibitors of tyrosine kinase with acceptable pharmacokinetic or ADME properties. The inhibitor inhibits the targetted structure to the C-terminal end of the A-loop, with the side of methionine chains occupying two hydrophobic pockets on the C-terminal lobe of the kinase. The synthetic compound, RBMS-01 was preceded and it recorded -44.000 Kcal as least energy.

Keywords: Insulin Receptor Tyrosine Kinase, Docking, Schrodinger, Maestro, Interactions.

INTRODUCTION

The hormone insulin stimulates the signalling intracellular pathways and it is regulating the cellular growth and its activities. The insulin mediates by its cell surface receptor (α,α,β,β) transmembrane glycoprotein by binding with intrinsic protein tyrosine kinase activity[1]. It induces the activity at β subunits. The autophosphorylation of Cytoplasmic kinase domain promotes the cascade of signalling events with the recruitment of specific substrates like IRS-1 and other phosphatidylinositol-3-kinase and glucose transporters to the cell surface. The cytoplasmic kinase domain region of the β subunit performed autophosprhorylation and also occurs in juxtamembrane, activation loop and COOH-terminus subdomains[2].

The crystallographic studies reveal that the Insulin receptor upon autophosphorylation, the kinase A-loop undergoes a major change in conformation. During unphosphorylated state *i.e.,* low activity form of Insulin Receptor Kinase Domain (IRKD) with Phosphate group which can prevent the substrate binding. The activated form of Tyr 1158, 1162 and 1163 involved in autophosphorylation and the tri-phosphorylated ATP in A-loop configuration stabilizes and makes binding sites of cofactors and substrates. The catalytic positions are properly placed.

Tyrosine Kinase

The developed small molecule acts by producing insulin dependent activation of Insulin receptor tyrosine kinase domains. The small molecule has the advantages which might resist to the physiological activity to hyperglycemia[3]. The large amount of receptor for tyrosine kinase includes one and three tyrosine residues in the kinase A-loop, which involves VII and VIII subdomains of the protein kinase catalytic core[4]. In the tyrosine kinase domain other than insulin receptor can be identified, it is also depend on tyrosine autophosphorylation in the A-loop which is Insulin like growth factor 1 (IGF-1) it is other than insulin receptor[5].

MATERIALS AND METHODS

ligand preparation

Ligands was obtained from Interbioscreen Ltd., (IBS), Moscow, Russia, a synthetic molecule with various functional groups. It is modified using ligprep module of the Maestro application, Schrodinger Inc, by assigning the appropriate bond orders manually. Each ligand was prepared manually with a full minimization with force field of the parameters using OPLS (Optimized potential for liquid simulations)[6] to eliminate bond length and angles biased from the crystal structure. The multiple structure for each ligand were produced with different combinations, so the protonation states *i.e.,* ionization states were exist to involve in any physiological condition. This operation can be performed using ionizer in Ligprep. Ligand, functions optimally in the pH range of 7. One or more forms of different conformations were produced to interact more strongly with the respective binding site. Tautomeric activity was also identified for the particular inhibitor.

Macromolecular preparation

The co-ordinates for all the proteins obtained from Research Colloboration for Structural Bioinformatics (RCSB, www.rcsb.org) (PDB ID 1IR3). The structure was derived from RCSB in which all the units contain same binding site for the ligand. In the present study has considered only one ligand. The complex was prepared by the module protein preparation wizard, where hydrogens were added automatically and refinement (Fig. 1) of the structure was also done. Water molecules were removed for the protein-ligand interactions and bond orders were re-assigned. The structure was minimized to a Root mean Square deviation of 0.30Å.

Ramachandran Plot Analysis

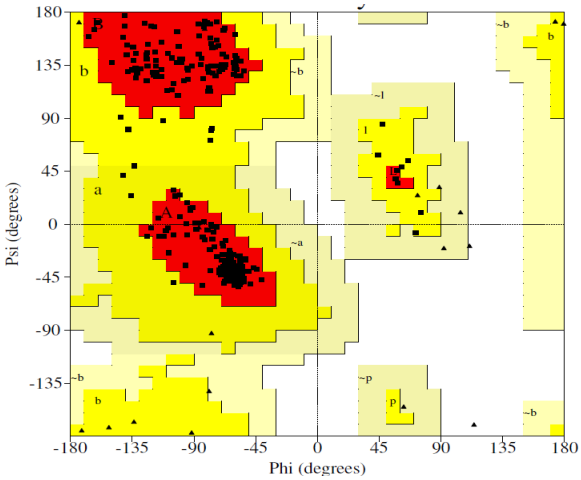

Fig. 1: The Ramachandran plot describes that most of the residues except glycine and proline were appeared in the allowed and favorable regions.

Ramachandran Plot analysis shows that most of the residues are presented in favorable regions. The glycine and proline are presented in disallowed regions. The regions presented in favorable regions are 93.9% and additionally allowed regions are 6.1%. It means that, this macromolecule can be utilized for further analysis.

Ligand docking parameters

Protein and ligands are used as the initial coordinates for docking process. The docking can be used in two approaches *i.e.,* by having ligand as flexible and receptor is rigid or ligand is rigid and receptor is flexible. The later approach *i.e.,* receptor rigid option is used for the present study, with the help of GLIDE docking approach[7].

The ligand docking was performed by the receptor grid generation; for this approach the macromolecular structure complexed with peptide substrate is taken. During the grid generation, no vander Waals radius sampling was done and the partial charge cut-off has been taken as 0.25 and no constraints were applied[8]. The location of peptide substrate has taken as binding site for all the other ligands for docking.

RESULTS AND DISCUSSION

Virtual Screening

After screening the library of taken natural and synthetic compounds, the score is sorted based on High Throughput Virtual Screening (HTVS). The study shows that synthetic compounds have better activity than the natural compound. Further the induced fit docking study is performed for the synthetic compounds.

The GLIDE docking method is applied to inhibitors to build an affinity model with the insulin receptor tyrosine kinase. The training sets (Fig.2) of different inhibitors are generated by scoring functions. It was characterized by orientations and Hydrogen bond positions. According to the high energy values, the synthetic molecule RBMS-01 has shown better activity with low root mean square (RMS) deviations of 0.30 Å. The cavity energy term is very small; it indicates that there is a low energy penalty when the ligand is buried in the cavity. These observations shows that inhibition of the ligand is depend on the various conformations with the rigid type of docking. In the best docked result, the synthetic molecule, named RBMS-01 has a best interaction with Asp, Tyr, Met, Asn of Insulin receptor tyrosine kinase.

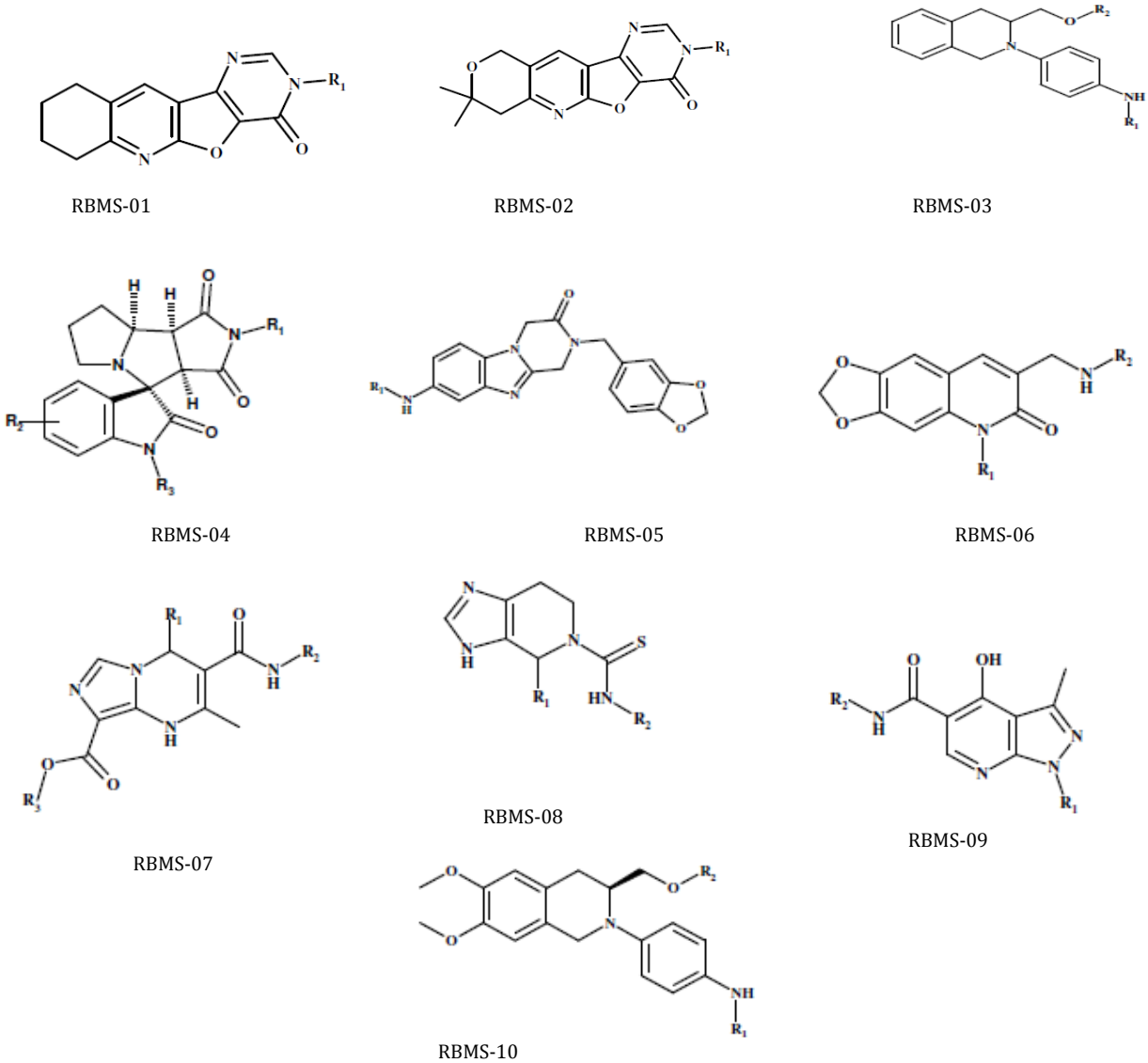

Fig 2: Chemical structure of different kinase inhibitors in their optimized positions. The structure were generated using Chemsketch (versions ACD Chemsketch 12.0).

The ligands are filtered by using High throughput virtual screening approaching using GLIDE docking, in which the High energy molecule were taken. During the docking process the ligands are rigid and receptor treated as rigid. All the inhibitors were passed through a scaling factor of 0.8 and partial charge cut-off of 0.15. The values were given in Table 1,

Table 1: HTVS results of Vander Waals (Evdw), Electrostatic (Ecoul), Docking score and energy values. Here, RBMS-01 shows better activity and interactions compared with other compounds.

Inhibitor	Evdw	Ecoul	Docking Score	Energy
RBMS-01	-14.54	-27.54	-9.40	-42.07
RBMS-02	-18.27	-23.78	-9.20	-42.05
RBMS-03	-15.87	-22.11	-9.03	-37.99
RBMS-04	-13.04	-37.16	-9.02	-50.19
RBMS-05	-09.12	-34.21	-9.02	-43.32
RBMS-06	-09.86	-39.55	-9.01	-49.41
RBMS-07	-09.65	-28.45	-8.99	-38.10
RBMS-08	-09.13	-24.76	-8.99	-33.89
RBMS-09	-10.15	-23.59	-8.94	-33.75
RBMS-10	-12.82	-30.65	-8.85	-43.47

Comparative Studies with natural compounds

The present study is extended to analyze the compound with the natural derivatives. The procedure carried out using HTVS. The lists of compounds are shown in Table 2.

Table 2: Comparitive studies of the natural compounds.

Compounds	Docking Score	Energy
Voglibiose	-6.14	-34.67
Phenformin	-5.60	-19.67
Miglitol	-4.08	-19.19
Metformin	-4.79	-15.78
Tolazamide	-3.14	-26.40
Gliclazide	-2.94	-26.96
Acetohexamide	-2.58	-24.21
Insulin like molecule	-4.97	-25.67

Results of energy and Glide Score. Among the analyzed voglibiose shown good affinity with the G-Score of -6.14 and Acetohexamide had a G-Score of -2.58. Insulin like molecule has the G-score of -4.97; it indicates that voglibiose has good affinity among the compared compounds.

Induced Fit Docking

The ligands were docked into binding site of the receptor where the receptor is rigid and the ligand is free to move, that has been filtered out from many compounds using Virtual screening[9]. The Insulin receptor shows the critical hinging and displays more conformations; it may allow side chain modelling. The conformations show that many alterations in the receptor and it is more closely conforms and fit to the ligands. The purpose of docking is to find the affinity between the macromolecular and ligand complex. Further, it shows the binding between the ligands into a rigid receptor, which assumes the correct one with low energy values. The purpose of this method is to eliminate the steric clashes, and then the appropriate interaction will be resulted. The IFD docking was performed from least energy after series of filtration from HTVS and standard precision using GLIDE docking. The synthetic compound, RBMS-01 was preceded and it recorded -44.000 Kcal as least energy. This reflects by sampling of the receptor degree of freedom and a minimization of the receptor-inhibitor complex for many different receptor poses and it is attempted to identify low free energy conformation of the each complex and many researchers demonstrated the importance of ligand binding with protein[10].

Loop Activation

Direction is most important criteria in terms of rotation, that towards the C-terminal lobe and it is parallel to the long axis of the molecule. While rotation taken place the interaction of Glu 1047 can be noticed with Lys 1030. The position of the activation loop was remodeled based on plausible backbone fragments of the same length extracted from Protein Data Bank (www.rcsb.org) and the given IRK sequence. An autophosphorylation taken place in the regions of Tyr 1158, 1162 and 1163, the regions of Asp, Lys, Met, Glu and Ser were found in active site residues which shows binding towards the ligand.

The Insulin receptor kinase structure, together with mutagenesis data shows the substitution of pTyr 1162 with phenylalanine increases the basal level kinase activity suggests that residues of the unphosphorylated A-loop compete with ATP and peptide substrate for binding at kinase acive site. Upon Insulin triggered trans-autophosphorylation of the tyrosine residues with the A-loop, this peptide segment adopts markedly different conformation which is stabilized by pTyr and non-Tyr interactions. An *in silico* autophosphorylation shows an evidence of stabilization of 'A'-loop conformation. Further it shows the activity of protein substrates and ATP as well as having an option of placing the residues critical for Mg-ATP binding its catalysis.

Autophosphorylation mapping experiments have indicated that Tyr1163 is the last of the three A-loop sites to be phosphorylated[11]. This result, together with the mutagenesis data and the structural results presented here, is consistent with a graded activation mechanism in which each autophosphorylation event in the A-loop leads to partial stabilization of the active configuration of the A-loop, with full activation achieved only upon autophosphorylation of pTyr1163. Conceivably, in partially activated states, pTyr1158 and pTyr1162 are engaged in a different set of interactions than those observed in the IRK structure. In this region, there is a structural substitution which makes less effective in the form of substitution for Tyr 1162 to Tyr 1163.

The pTyr1162 and Arg1164 pair serves docking sites for downstream signalling proteins. Several recent studies state that one or more loop of pTyr residues are actively involved in protein-ligand, protein-protein interactions

Functional inference

The parent compound (RBMS-01) shows the good affinity with the macromolecular and also the derivative of the parent compound taken as an account and also shows the evidence of better binding affinity with the macromolecular structure. The present compound was initiated to explore the ability and to develop an inhibitor by mimicking the above mentioned interactions. The α-C makes the proper rotation and it is configured active site on the positions of Asp, Lys, Met, Glu and Ser.

With the literature evidence, many inhibitors of tyrosine kinase have been reported. The presented synthetic compound shows the better binding affinity and energy values from the Induced Fit Model. The ligand binding site of intracellular C-terminal region displays the highest level of conservation and comprises catalytic domains responsible for the kinase activity of these receptors, which catalyses receptor autophosphorylation and tyrosine phosphorylation. The inhibitor inhibits the targetted structure to the C-terminal end of the A-loop, with the side of methionine chains occupying two hydrophobic pockets on the C-terminal lobe of the kinase. It shows that the above mentioned compounds induces the insulin action after it binds with insulin receptor eventually leads to an increase in the high affinity glucose transporter (Glut4) molecules on the outer membrane of insulin-responsive tissues, including muscle cells and adipose tissue, and therefore to an increase in the uptake of glucose from blood into these tissues.

ADME or pharmacokinetic predictions of the best fit molecules

The ligands with the comparable scores with other molecules were subjected to predict pharmacokinetic properties using the QikProp module of the software. QikProp settings determine which molecules are flagged as being dissimilar to other 95% of the known drugs. Predicted significant ADME properties such as permeability through the predicted log IC50 value for blockage of K+ channels (QPlogHERG), QikProp predicted gut-blood barrier and no violations of Lipinski's rule of five are reported here (Fig. 3). The predicted property of docked compound described in Table 3.

Table 3: Information about Ligand. The drug molecule which satisfies Lipinski rule of 5 includes molecular weight, hydrogen bond donors and acceptors.

Compound	HTVS	
	G Score	Energy
RBMS-01-DER1	-4.17	-32.91
RBMS-01-DER2	-3.88	-29.32

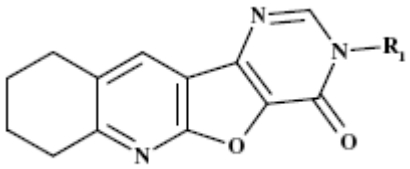

Fig 3: Structure of Ligand molecule, parent molecule structure of RBMS-01.

Ligplot Analysis

The analysis shows the pattern of interactions protein-ligand complexes and any metal ions (Fig. 4), which it binds, allowing a fast analysis of the location of specific intermolecular interactions with respect to the sequence.

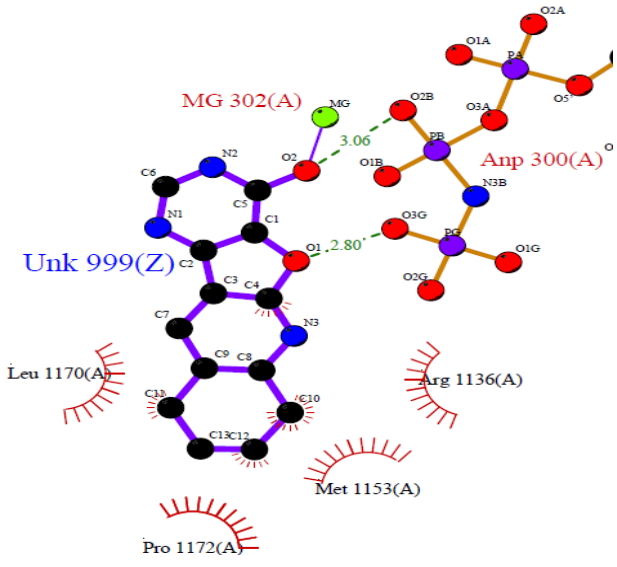

Fig 4: Protein-Ligand Interaction using Ligplot, which shows the better interaction with Leucine, Methionine, Arginine and cofactor Mg^{2+}.

The LIGPLOT program which automatically generates schematic 2-D representations of protein-ligand complexes from standard PDB file input. This analysis showed the better interaction between the ligand and macromolecule. It represents the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities.

Interactional Analysis

The derived macromolecule insulin receptor kinase was docked with novel synthetic molecule and was found to have significant affinity (Table 4) (Figure 5 and 6).

Docking representation

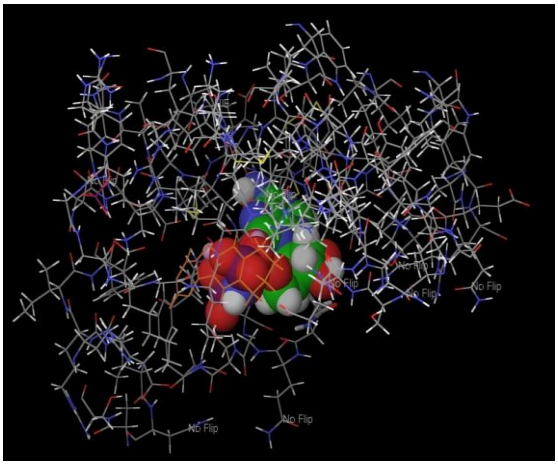

Fig 5: The representation of docked molecule between 1IR3 and ligand in wireframe representation.

Protein-Ligand Interaction

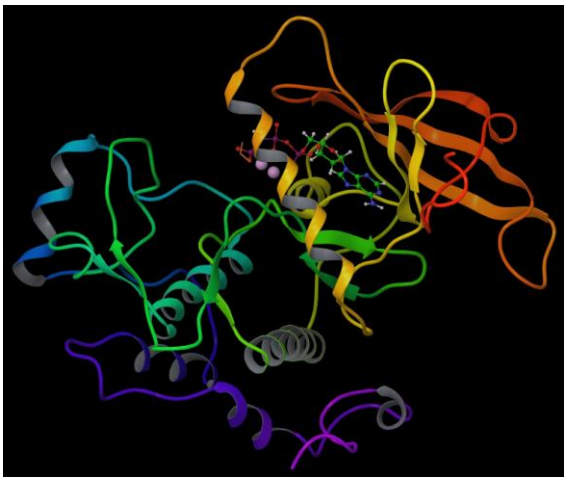

Fig 6: The representation of docked molecule between 1IR3 and ligand in wireframe representation.

Table 4: The Table describes about the interactional analysis between ligand and macromolecule where serine, glutamate shows the better activity.

Contents	Values
Compound Name	RBMS-01
Molecular Weight	241.249
Octanol/Water	1.824
Log IC_{50}	-3.874
Lipinski's violations	Nil
QlogP MDCK	332

Derivatives of the parent molecule

The compound RBMS-01 produces good docking and energy scores when compare to other compounds. The der-1 can be derived from RBMS-01 by introducing benzene derivative in the 3rd position. The –NH group shares with the dimethyl group and another is ketone group with heterocyclic compound. The substituted ring shows the better activity which displayed in (Fig 7) and GLIDE score and Energy values are mentioned in Table 5

Fig 7a: RBMS-01-der-1 ,b. RBMS-01-der-2. The structure derived in 3rd position of the ring. The molecule RBMS-01-der-1 shown better activity comparatively.

Table-5: G-score and Energy value of Derivatives of RBMS-01. RBMS-02-DER2 stabilizes the G-Score and Energy value doing in HTVS. Then it shows a better interaction with selected residues.

D .. H .. A	Dist in Å	G Score	Energy in Kcal
UnK H-H-O Asp(1083)	1.838	-9.7387	-45.47
Lys (1030) H-H-O Unk)	2.367	-9.7387	-45.47
Met (1079) H-H-O(Unk)	2.236	-9.7387	-45.47
Unk N -H-O(Glu 1077)	1.811	-9.7387	-45.47
Ser1006 H-H-O(Unk)	1.600	-9.7387	-45.47
Ser 1006 H - H – O1B (UNK)	1.572	-9.7387	-45.47

CONCLUSION

Comparative studies conclude that the proposed inhibitor represents a new generation of drugs for the trea tment of Type II diabetes. Earlier studies of various drug molecules like Acetohexamide, metformin *etc.* also examined. Most of these compounds have different molecular structures and possess specific mode of action. The analyzed synthetic molecule which shows the better activity compared with already existing natural drugs. Present study confirms that the natural and synthetic molecule shows the potentiality in the hydrophobic effect based on GLIDE energy values and the followed active sites. The proposed inhibitor which might be more effective since it simulates the residual interaction with the respective residues like Aspartic Acid, Lysine, Methionine, Glutamic acid and Serine also it satisfies the properties of Lipinski's rule of 5. Hence, the proposed drug can be used for further scientific studies and to be extended to experimental validation.

Acknowledgement

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